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Effects of IL-13 on Phenotype, Cytokine Production, and Cytotoxic Function of Human Monocytes

Comparison with IL-4 and Modulation by IFN- γ or IL-10¹

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ABSTRACT. Recently, we described the cloning and expression of a human cDNA which is the homologue to P600, a gene transcribed by mouse Th2 clones. Based on its activities on human monocytes and B cells this gene was designated IL-13. In the present study we investigated the effects of IL-13 alone or in combination with IL-4, IFN- γ , or IL-10 on human monocytes. IL-13 induced significant changes in the phenotype of monocytes. Like IL-4, it enhanced the expression of CD11b, CD11c, CD18, CD29, CD49e (VLA-5), class II MHC, CD13, and CD23, whereas it decreased the expression of CD64, CD32, CD16, and CD14 in a dose-dependent manner. IL-13 induced up-regulation of class II MHC Ag and its down-regulatory effects on CD64, CD32, and CD16 expression were prevented by IL-10. IFN- γ could also partially prevent the IL-13-induced down-regulation of CD64, but not that of CD32 and CD16. However, IL-13 strongly inhibited spontaneous and IL-10- or IFN- γ -induced ADCC activity of human monocytes toward anti-D coated Rh⁺ erythrocytes, indicating that the cytotoxic activity of monocytes was inhibited. Furthermore, IL-13 inhibited production of IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-12 p35, IL-12 p40, macrophage inflammatory protein-1 α , granulocyte/macrophage-CSF, granulocyte-CSF, IFN- α , and TNF α by monocytes activated with LPS. In contrast, IL-13 enhanced the production of IL-1ra by these cells. Similar results on cytokine production were observed or have been obtained with IL-4. Thus IL-13 shares most of its activities on human monocytes with IL-4, but no additive or synergistic effects of IL-4 and IL-13 on human monocytes were observed, suggesting that these cytokines may share common receptor components. Taken together, these results indicate that IL-13 has anti-inflammatory and important immunoregulatory activities. *Journal of Immunology*, 1993, 151: 6370.

Activated T cells secrete a number of biologically active polypeptides, which regulate the proliferation, differentiation, and function of cells participating in immune responses against Ag (1). T cells pro-

ducing IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IFN- γ , GM-CSF,³ and TNF/LT simultaneously after antigenic or polyclonal stimulation have been described in both mouse and man (2–4). These T helper cells were designated Th0 cells to distinguish them from the more specialized Th1 and

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³ Abbreviations used in this paper: GM, granulocyte/macrophage; G, granulocyte; HSA, human serum albumin; MIP, macrophage inflammatory protein; PCR, polymerase chain reaction; nt, nucleotide; ADCC, antibody-dependent cell-mediated cytotoxicity.

Th2 subsets (5). Murine Th1 cells produce IL-2, IFN- γ , TNF/LT, IL-3, and GM-CSF which support their function as regulatory and effector cells in cellular immune responses such as delayed-type hypersensitivity, whereas Th2 cells produce IL-4, IL-5, IL-6, IL-10, IL-3, and GM-CSF which make them suitable for providing help to B cells in the production of immunoglobulins of different isotypes (6, 7). In humans, T cell clones with restricted cytokine production profiles have also been isolated from patients with infectious or allergic diseases (8–12). Although these type of clones resembled murine Th1 and Th2 clones, there were some differences. Depending on their mode of activation, Th1 clones generally could still produce low quantities of IL-4, whereas Th2 clones were able to produce low to normal quantities of IFN- γ (10–12). However, a clear imbalance in the production ratios of IL-4 and IFN- γ by Th2 clones was observed after antigenic stimulation (12). Therefore we defined human T cell clones which produce high levels of IFN- γ and no or low levels of IL-4 Th1-“like” cells and T cell clones which produce no or low levels of IFN- γ and high levels of IL-4 Th2-“like” cells (10–12). Furthermore, IL-10 which is exclusively produced by Th0 and Th2 T cell subsets in the mouse is synthesized by Th0, Th1-“like,” and Th2-“like” subsets in humans (13).

Recently, we and others (14) cloned the human cDNA homologue of P600, a mRNA which is transcribed by activated mouse Th2 clones (15, 16) and expressed the mouse and human cDNA clones in *Escherichia coli* (17). Both human and mouse P600 proteins were biologically active and affected human monocyte and B cell functions. Therefore, we proposed that this novel cytokine be designated IL-13 (17). Human IL-13 is a unglycosylated protein of 132 amino acids with a molecular mass of 10 kDa. Both human and mouse IL-13 induced proliferation of the human premyeloid cell line TF1. In addition, IL-13 was shown to induce changes in the morphology of the adherent fraction of human PBL which contained predominantly monocytes. These cells formed long cellular processes and adhered strongly to the substrate. IL-13 also induced changes in the phenotype of human monocytes and human B cells by up-regulating class II MHC expression and inducing the expression of the low affinity receptor for IgE (Fc ϵ RII, CD23) (17, 18). Furthermore, IL-13 acted on human B cells by inducing proliferation and immunoglobulin production. In particular, IL-13 induced human B cells to switch to ϵ and produce IgG4 and IgE in the presence of T cell clones, T cell membranes, or CD40 ligand (18, 19). These results indicated that many of the presently known biologic activities of IL-13 are shared with IL-4.

In this study the biologic activities of mouse and human IL-13 on human monocytes were further characterized and compared with those of IL-4, IL-10 and IFN- γ , other cytokines with stimulatory or inhibitory actions on human monocytes. IL-13 induced dramatic changes in the pheno-

type of human monocytes and inhibited the production and expression of IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-12 p35, IL-12 p40, GM-CSF, G-CSF, MIP-1 α , and TNF α after activation by LPS, whereas it enhanced the production of IL-1ra. These results indicate that IL-13 has anti-inflammatory activities and may play an important regulatory role in immune responses.

Materials and Methods

Isolation and culture of human monocytes

Human monocytes were isolated from peripheral blood of healthy donors by centrifugation over Ficoll-Hypaque and adherence to plastic. Briefly, 100×10^6 PBMNC were plated on a 100-mm tissue culture dish in Yssel's medium (20) supplemented with HSA and 1% pooled human AB⁺ serum and incubated at 37°C for 30 min. This culture medium was endotoxin free as determined by the Limulus amoebocyte lysate assay (<0.2 ng/ml of endotoxin). Subsequently, nonadherent cells were removed by extensive washing and cultured in Yssel's medium with HSA and 1% pooled human AB serum as indicated. Alternatively, highly purified human peripheral blood monocytes were obtained from 500 ml of blood of normal donors by centrifugal elutriation as described previously (21, 22). Mononuclear cells were isolated by density centrifugation in a blood component separator, followed by fractionation into lymphocytes and monocytes. The monocyte preparation was $>95\%$ pure, as judged by nonspecific esterase staining, and contained more than 98% viable cells. These monocytes were cultured in Yssel's medium with HSA and 1% pooled human AB⁺ serum at a concentration of 4×10^6 cells/ml in Teflon bags (Jansen MNL, St. Niklaas, Belgium), which prevented adhesion of these cells. After culture for the times indicated, monocytes were collected and analyzed for cell surface expression by indirect immunofluorescence or analyzed for lymphokine gene expression by Northern and PCR analysis. In addition, monocyte culture supernatants were collected for determination of IL-1 α , IL-1 β , IL-6, IL-8, IL-10, TNF α , MIP-1 α , GM-CSF, G-CSF, and IL-1ra production after activation of these cells by LPS (*E. coli* 0127:B8) (Difco, Detroit, MI) at 1 μ g/ml. The viability of the cells after culture always exceeded 95% as determined by trypan blue exclusion.

Reagents

Human and mouse rIL-13 were expressed in *E. coli* as insoluble aggregates of glutathione-S-transferase fusion proteins, extracted by centrifugation, solubilized, and subjected to renaturation before digestion with thrombin to remove the N-terminal fusion part (23). Subsequently, proteins were purified by cation exchange and gel filtration chromatography, which resulted in active human and mouse IL-13 as described previously (17). Purified human

rIL-10, rIL-4, and rIFN- γ were provided by Schering-Plough Research (Bloomfield, NJ). The neutralizing anti-IL-4 mAb 25D2 (24) and anti-IL-10 mAb 19F1 (25) were described previously. The following mAb were used for immunofluorescence studies on the expression of cell surface markers: SPV-L7 (CD11a) (26), Bear-1 (CD11b) (27), CLB FcR gran-1 (CD16) (28), mAb 25 (CD23) (29), IV.3 (CD32) (30), 32.2 (CD64) (31), Q5/13 (HLA-DR/DP) (32), PdV5.2 (HLA-DR/DP/DQ) (33), SAM-1 (VLA-5, CD49e) (34), and L307 (B7) (35). IOM13 (CD13) and CD29 were purchased from AMAC, Inc (Westbrook, ME). Leu-M3 (CD14), Leu-M5 (CD11c), and L130 (CD18) were obtained from Becton-Dickinson (San Jose, CA).

Probes

Oligonucleotides used for Southern analysis of IL-1 α , IL-1 β , IL-6, GM-CSF, G-CSF, and β -actin PCR products and DNA fragments used for detection of IL-8, IL-10, TGF- β , and β -actin mRNA have been described (36). The following oligonucleotides were used to detect IFN- α , 5'-TTCTGGCTGTGAGGAAATACT-3' (nt 360-378) (37); IL-12 p35, 5'-AATGGGAGTTGCCTGGCCTC-3' (nt 488-507) (38); IL-12 p40, 5'-TAAGACCTTTCTAAGATGCGAGGCC-3' (nt 417-441) (38).

mRNA isolation and northern analysis

Total RNA was isolated from 20×10^6 monocytes by the guanidinium thiocyanate-CsCl procedure (39). For Northern analysis, 5 μ g total RNA per sample was separated according to size on 1% agarose gels containing 6.6% formaldehyde, transferred to Nytran nylon membranes (Schleicher & Schuell, Keene, NH), and hybridized with probes, labeled to high specific activity ($>10^8$ cpm/mg) by the hexamer labeling technique (40). Filters were hybridized, washed under stringent conditions, and developed as described previously (41).

PCR analysis

One microgram of total RNA was reverse transcribed using oligo(dT)₁₂₋₁₈ as primer (Boehringer Mannheim, Indianapolis, IN) and superscript reverse transcriptase (GIBCO BRL, Gaithersburg, MD) according to the procedure of Krug and Berger (42) in a 20- μ l reaction. Two microliters of reverse transcript (equivalent to 100 ng of total RNA) was used directly for each amplification reaction. Conditions for PCR were as follows: in a 50- μ l reaction, 25 nmol of each primer, 125 μ M each of dGTP, dATP, dCTP, and dTTP (Pharmacia, Uppsala, Sweden), 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl₂, 1 mg/ml gelatin, 100 mg/ml nonacetylated BSA, and 1 U of Vent DNA polymerase (New England Biolabs, Beverly, MA). Primers used to amplify IL-1 α , IL-1 β , IL-6, GM-CSF, G-CSF, and β -actin have been described previously (36). The following

primers were also used: IFN- α sense primer, 5'-GCT-GAAACCATCCCTGTC-3' (nt 161-178) (37); IFN- α antisense primer, 5'-CTGCTCTGACAACCTCCCAG-3' (nt 450-430) (37); IL-12 p35 sense primer, 5'-CTTCACCACTCCCAAACCTG-3' (nt 281-302) (38); IL-12 p35 antisense primer, 5'-AGCTCGTCACTCTGTCAATAG-3' (nt 813-792) (38); IL-12 p40 sense primer, 5'-CATTCGCTCCTGCTGCTTCAC-3' (nt 337-358) (38); IL-12 p40 antisense primer, 5'-TACTCCTTGTTGTCCCCTCTG-3' (nt 603-582) (38). Reactions were incubated in a Perkin-Elmer Cetus DNA Thermal cycler 9600 for 25 cycles (denaturation 30 s 94°C, annealing 30 s 55°C, extension 60 s 72°C). Forty microliters of each reaction was loaded on 1% agarose gels in TAE buffer and PCR products were visualized by ethidium bromide staining. Subsequently, gels were denatured in 0.5 M NaOH, 1.5 M NaCl, neutralized in 1 M ammonium acetate, and transferred to Nytran nylon membranes. Membranes were prehybridized in $6 \times$ SSC, 1% SDS, $10 \times$ Denhardt's solution (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% BSA, pentax fraction V) and 200 mg/ml *E. coli* tRNA (Boehringer, Mannheim, FRG) for 4 h at 55°C. Oligonucleotide probes (200 ng), specific for a sequence internal to the primers used in the amplification, were labeled at the 5' end by T4 polynucleotide kinase (New England Biolabs) and [γ -³²P]ATP (Amersham, Arlington Heights, IL) as described (36). Probes were separated from nonincorporated nucleotides by passage over a Nick column (Pharmacia, Uppsala, Sweden) and added to the hybridization mixture. After hybridization for 12 h at 55°C, filters were washed in $0.1 \times$ SSC ($1 \times$ SSC: 150 mM NaCl, 15 mM sodium citrate, pH = 7.0), 0.1% SDS at room temperature, and exposed to Kodak XAR-5 films for 1 to 2 h. In addition, signals were quantified on a Molecular Dynamics phosphor-imager (Molecular Dynamics, Sunnyvale, CA).

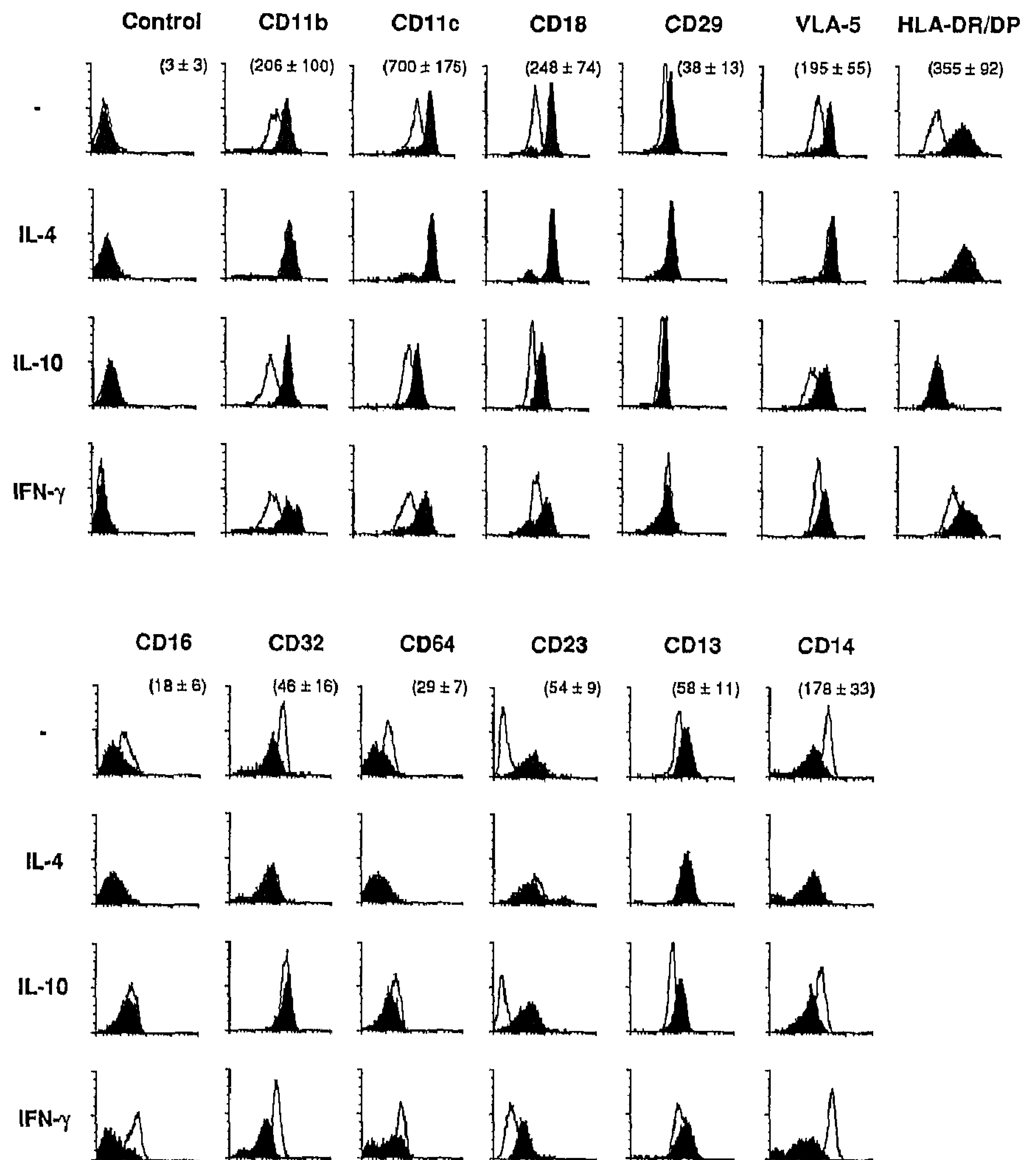
Lymphokine determinations

The production of cytokines by monocytes was determined in culture supernatants by cytokine-specific ELISA which were either obtained commercially or described previously. The cytokine-specific ELISA and their sensitivities were: IL-1 α Endogen (Boston, MA) (50 pg/ml), TNF α Endogen (Boston, MA) (10 pg/ml), IL-1 β Cistron (Pine Brook, NJ) (20 pg/ml), IL-6 Genzyme (Boston, MA) (0.313 ng/ml), IL-8 R&D Systems (Minneapolis, MN) (4.7 pg/ml), G-CSF R&D Systems (Minneapolis, MN) (7.2 pg/ml), IL-1ra R&D Systems (Minneapolis, MN) (12.5 pg/ml), MIP-1 α R&D Systems (Minneapolis, MN) (2.0 pg/ml), GM-CSF (43) (50 pg/ml), and IL-10 (25) (75 pg/ml).

Immunofluorescence analysis

Cells (10^5) were incubated in V bottom microtiter plates (Flow Laboratories, McLean, VA) with 10 μ l of purified mAb (1 mg/ml) for 30 min at 4°C. After two washes with

FIGURE 1. Effect of cytokines on the expression of cell surface Ag by monocytes. Human monocytes were cultured in medium, IL-4 (400 U/ml), IL-10 (200 U/ml), or IFN- γ (100 U/ml) in the (□) absence or the (■) presence of IL-13 (50 ng/ml) for 120 h, and the expression of cell surface Ag was determined by indirect immunofluorescence. The mean changes in mean fluorescence intensity \pm SD of six individual experiments are given in parentheses.



PBS containing 0.02 mM sodium azide and 1% BSA (Sigma), the cells were incubated with 1:40 dilution of FITC-labeled F(ab')₂ fragments of goat anti-mouse antibody (TAGO, Inc. Burlingame, CA) for 30 min at 4°C. After three additional washes, the labeled cell samples were analyzed by flow microfluometry on a FACScan (Becton Dickinson, Sunnyvale, CA).

ADCC

ADCC activity of cultured human monocytes against antibody coated rhesus positive human erythrocytes was performed as previously described (44).

Results

IL-13 and IL-4 induce identical changes in monocyte surface Ag expression

Previously, we described that both mouse and human IL-13 induced expression of CD23 (FcεRII) and up-regulated the expression of class II MHC Ag on human monocytes. The effects of IL-13 on the expression of a larger panel of cell surface Ag was examined here. A representative experiment shown in Figure 1 indicated that IL-13 affected the

expression of multiple cell surface molecules belonging to different supergene families. The mean changes in fluorescence intensity by IL-13 from six independent experiments are given in parentheses. IL-13 enhanced the expression of several members of the integrin superfamily of adhesion molecules. The expression of the subunits CD11b (C3bi receptor, Mac-1), CD11c (gp150), and VLA-5 (FNR) as well as their respective β subunits CD18 (β 2) and CD29 (β 1, VLA-b) was up-regulated by IL-13. The expression of other members of this family, including CD11a (LFA-1), VLA-2 (CD49b), VLA-3, VLA-4 (CD49d), VLA-6 (CD49f), β 3 (CD61) and β 4 was not significantly affected by IL-13 (not shown).

IL-13 enhanced the expression of class II MHC Ag (Fig. 1). The expression of HLA-DR, HLA-DP, and HLA-DQ was up-regulated by IL-13. Expression of other members of the immunoglobulin superfamily including class I MHC, CD11a(LFA-1), CD54 (ICAM-1), ICAM-2, and CD58 (LFA-3) was not affected by IL-13 (not shown).

IL-13 modulated the expression of the various Fc receptors on monocytes. The expression of CD64 (FcγRI), CD32 (FcγRII), and CD16 (FcγRIII) on human monocytes was strongly down-regulated by IL-13 (Fig. 1). In contrast,

Table I
IL-13 acts independently of IL-4^a

| mAb | Medium | IL-13 | | IL-4 | |
|--------------|----------------|-------------|------|-------------|-----|
| | | + anti-IL-4 | | + anti-IL-4 | |
| Control | 3 ^b | 5 | 6 | 5 | 3 |
| MHC class II | 443 | 1904 | 1845 | 2084 | 220 |
| CD23 | 3 | 99 | 79 | 89 | 8 |
| CD14 | 222 | 97 | 83 | 80 | 444 |

^a Monocytes were incubated with medium, IL-13 (50 ng/ml), or IL-4 (400 U/ml) in the absence or presence of neutralizing anti-IL-4 mAb 25D2 (10 µg/ml) at 37°C for 120 h; and expression of HLA-DR/DP (Q5/13), CD23 (mAb 25), and CD14 (Leu-M3) was determined by indirect immunofluorescence.

^b Mean fluorescence intensity (channel number).

as has already been demonstrated previously, IL-13 induced the expression of CD23 (FcεRII) (17). In addition, IL-13 up-regulated the expression of CD13 (aminopeptidase N) and down-regulated the expression of CD14 (Fig. 1). No effect of IL-13 was detected on the expression of CD25, CD33, and CD44 (not shown).

IL-4 up-regulated expression of CD11b, CD11c, CD18, VLA-5, CD29, class II MHC, CD13, and CD23 and inhibited the expression of CD16, CD32, CD64, and CD14 on human monocytes to the same extent as did IL-13 (Fig. 1). Changes in the expression of these markers by IL-13 or IL-4 were dose-dependent (not shown). Taken together, these data indicate that the IL-13-induced changes in the expression of cell surface molecules are identical with those induced by IL-4 (Fig. 1). Interestingly, incubation of monocytes with saturating concentrations of both IL-4 and IL-13 did not result in additive or synergistic activities effects on the expression of the various cell surface molecules (Fig. 1). To date, no evidence has been presented that monocytes are able to produce IL-4. However, to exclude the possibility that IL-13 acted through the induction of IL-4 by monocytes or by few contaminating T cells, monocytes were incubated in the presence of IL-13 and a neutralizing anti-IL-4 mAb. As shown in Table I, the induction of CD23, down-regulation of CD14 and up-regulation of class II MHC by IL-13 was not affected by the anti-IL-4 mAb. The anti-IL-4 mAb was effective because it completely inhibited the effects of IL-4 in control experiments (Table I). Thus, IL-13 acts independently of IL-4.

IL-10 down-regulates IL-13-induced class II MHC expression on human monocytes

To compare the effects of IL-13 with those of other cytokines which modulate the monocyte phenotype, these cells were incubated with IL-10 or IFN-γ in the absence or presence of IL-13, and the expression of cell surface Ag was analyzed. As shown in Figure 1, IL-10 or IFN-γ alone did not significantly affect the expression of CD11b, CD11c, CD18, CD13, CD23, CD29, and VLA-5. In addition, IL-10 or IFN-γ did not affect the IL-13-induced increase in expression of these markers. IL-10 or IFN-γ had also no effect

Table II
IL-10 inhibits constitutive, IL-13-, IL-4-, and IFN-γ-induced MHC class II expression on human monocytes^a

| Incubation | IL-10 (200 U/ml) | |
|-------------|------------------|-----------------|
| | - | + |
| Control 4°C | 69 ^b | ND ^c |
| Medium 37°C | 150 | 46 |
| mIL-13 | 212 | 73 |
| hIL-13 | 197 | 81 |
| IL-4 | 407 | 94 |
| IFN-γ | 347 | 36 |

^a Elutriated monocytes were incubated in medium at 4°C or 37°C, mouse IL-13 (50 ng/ml), human IL-13 (50 ng/ml), IL-4 (400 U/ml), or IFN-γ (100 U/ml) in the absence or presence of IL-10 (200 U/ml) in Teflon bags for 48 h; and expression of HLA-DR/DP was determined by indirect immunofluorescence.

^b Mean fluorescence intensity (channel number).

^c ND, not done.

on the expression of CD14 and the IL-13-induced inhibition of CD14 expression. However, IL-10 down-regulated not only the constitutive class II MHC Ag expression on monocytes (36), but also inhibited strongly the IL-13-induced class II MHC expression. Similar data were obtained when highly purified monocytes isolated by elutriation and cultured in Teflon bags were used (Table II). An increased expression of class II MHC Ag was observed after incubation of monocytes in medium alone, which was completely prevented by IL-10. mIL-13, hIL-13, IL-4, and IFN-γ all induced high levels of class II MHC expression which were blocked by IL-10 (Table II). Class II MHC expression induced by IFN-γ was further enhanced by IL-13 (Fig. 1). Taken together, these results indicate that IL-13, IL-10, and IFN-γ independently modulate the expression of monocyte surface Ag.

IL-13 inhibits monocyte FcγR surface expression and cytotoxicity

IFN-γ, IL-4, and IL-10 are able to modulate the expression of FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16) on human monocytes. IFN-γ and IL-10 enhance the expression of CD64, whereas IL-4 down-regulates the expression of CD64, CD32, and CD16 (Fig. 1) (44–46). Addition of combinations of these cytokines to monocytes showed that IL-10 was able to prevent the IL-4-induced down-regulation in cell surface expression of all three FcγR (44) and that IFN-γ partially restored the down-regulatory effects of IL-4 on CD64 expression (46). The effects of IL-10 and IFN-γ on IL-13-induced reduction in the expression of the various FcγR are shown in Figure 1. IL-10 prevented down-regulation of CD64, CD32, and CD16 induced by IL-13. In addition, IFN-γ could partially rescue IL-13-induced down-regulation of CD64, but did not affect the IL-13-induced down-regulation of CD32 and CD16 (Fig. 1).

The level of ADCC activity of human monocytes has been shown to correlate with the expression of FcγRI (47).

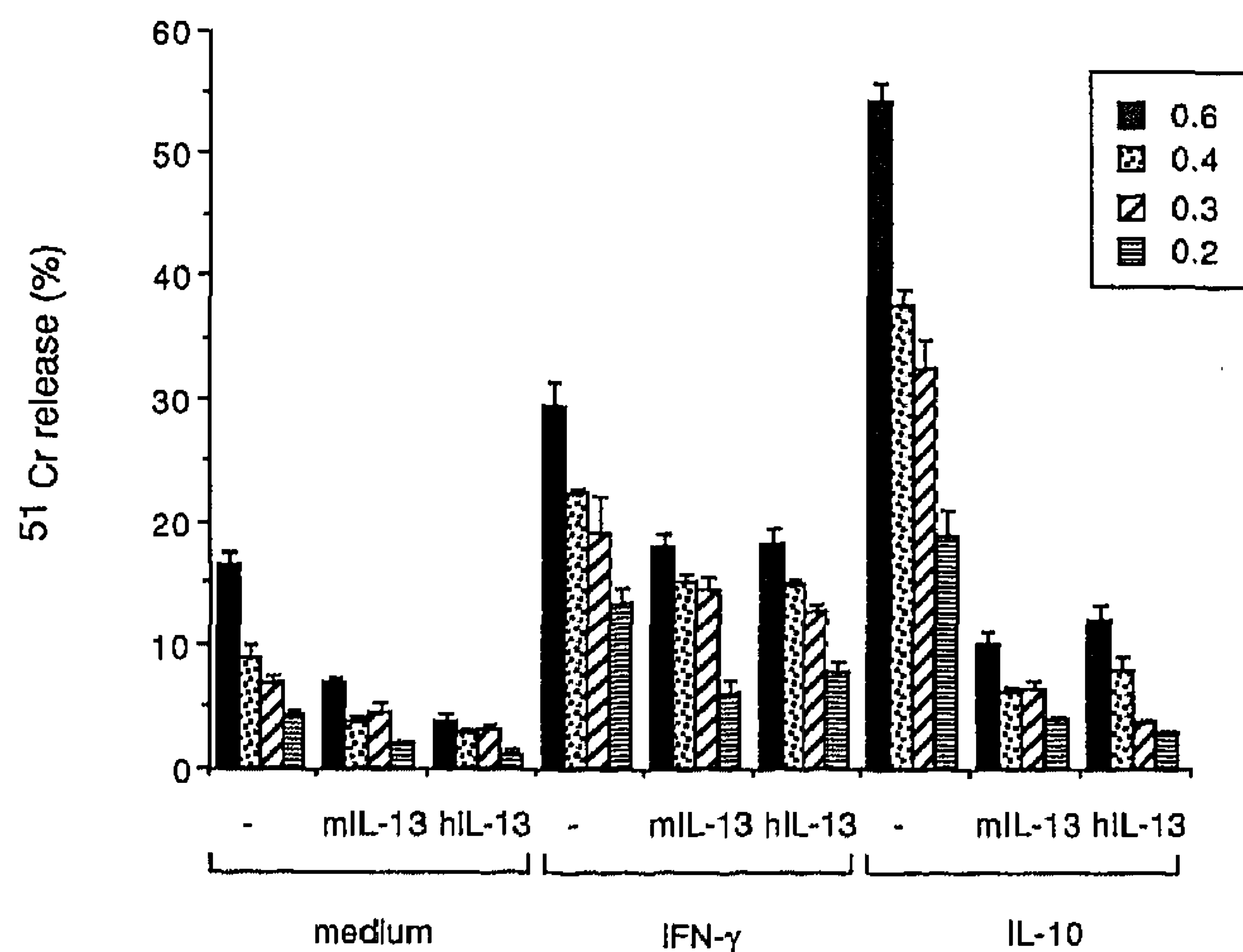


FIGURE 2. IL-13 inhibits spontaneous, IFN- γ - and IL-10-induced ADCC activity by monocytes. Elutriated monocytes (4×10^6 /ml) were cultured in medium, IL-10 (200 U/ml) or IFN- γ (100 U/ml) in the absence or presence of human (*h*) or mouse (*m*) IL-13 (50 ng/ml) for 40 h and ADCC activity against anti-D coated Rhesus⁺ human erythrocytes was determined at an effector/target ratio of 0.6, 0.4, 0.3, and 0.2.

Therefore, the effects of IL-13 on the functional activity of Fc γ RI on monocytes was determined by their ability to lyse anti-D opsonized human Rh⁺ erythrocytes. As shown in Figure 2 both human and mouse IL-13 were able to inhibit ADCC activity of monocytes cultured in medium alone. On the other hand, ADCC activity was enhanced when monocytes were cultured in the presence of IFN- γ or IL-10. Interestingly, IL-13 significantly inhibited these effects of IFN- γ and IL-10 despite the fact that IFN- γ and IL-10 partially or completely reversed the inhibition of Fc γ RI expression (Fig. 1), indicating that there is no absolute correlation between Fc γ RI expression and ADCC activity and that IL-13 affected the Fc γ R-mediated cytotoxicity also by other mechanisms.

IL-13 inhibits production of proinflammatory cytokines, chemokines and hematopoietic growth factors, but enhances IL-1ra secretion

The effects of IL-13 on the production of cytokines by human monocytes was investigated next. Monocytes were activated by LPS and cytokine production was determined in culture supernatants harvested after 20 h by cytokine-specific ELISA. Activation of monocytes by LPS resulted in the production of IL-1 α , IL-1 β , IL-6, IL-8, IL-10, G-CSF, TNF α , MIP1 α , and IL-1ra (Table III). IL-13 strongly inhibited the production of IL-1 α , IL-1 β , IL-6, IL-8, IL-10, G-CSF, and TNF α . As described previously, IL-4 and IL-10 also strongly inhibited the production of these cytokines (36, 48). Interestingly, IL-13, IL-4, and IL-10 strongly inhibited the production of MIP-1 α -like IL-8, a member of the chemokine family (49, 50). In contrast, IL-13, IL-4, and IL-10 enhanced the production of

IL-1ra. Monocytes are able to produce large amounts of IL-10 which can suppress the production of IL-1 α , IL-1 β , IL-6, IL-8, IL-10, GM-CSF, G-CSF, and TNF α in an autoregulatory fashion (36). It is shown that LPS-activated monocytes produced high levels of IL-10 in this particular experiment (Table III). To exclude the effects of endogenous IL-10, monocytes were also activated by LPS in the presence of neutralizing anti-IL-10 mAb 19F1. As shown in Table III, activation of monocytes in the presence of anti-IL-10 mAbs resulted in strongly enhanced production of IL-1 α , IL-1 β , GM-CSF, G-CSF, TNF α , and MIP-1 α , whereas IL-6 and IL-8 production was moderately enhanced. The production of IL-1ra was slightly inhibited by anti-IL-10 mAb. To examine the effects of IL-4 and IL-13 on cytokine production in the absence of endogenous IL-10, monocytes were activated by LPS in the presence of the anti-IL-10 mAb 19F1 and IL-4 or IL-13. As shown in Table III, IL-13 and IL-4 strongly inhibited the production of IL-1 α , IL-1 β , IL-6, GM-CSF, G-CSF, and TNF α . IL-4 also strongly inhibited the production of IL-8 and MIP-1 α under these conditions, whereas the inhibition of IL-8 and MIP-1 α by IL-13 was less pronounced.

The production of IL-1ra was enhanced by IL-4 and IL-13 as compared with activation by LPS and anti-IL-10 mAb, but was reduced in comparison with activation by LPS and either IL-4 or IL-13 in the absence of the anti-IL-10 mAb.

mRNA analyses by comparative PCR or Northern blotting confirmed the effects IL-4, IL-13, IL-10, and anti-IL-10 on the production of most cytokines by LPS-activated monocytes at the level of gene expression as shown in Figures 3 and 4. Generally, a good correlation was observed between the levels of mRNA or amplified cDNA product and secreted proteins with exception of IL-1 β . LPS induced the expression of IL-10 mRNA, which was inhibited by IL-4, IL-13, and IL-10. Activation of monocytes by LPS in the presence of anti-IL-10 mAb showed a strong increase in the expression of IL-10 mRNA as described previously (36), and under these conditions IL-4 and IL-13 were able to strongly inhibit IL-10 mRNA expression (Fig. 4). Levels of TGF β mRNA were not affected by activation of monocytes by LPS; and IL-4, IL-10, and IL-13 did not change this constitutive expression (Fig. 4).

The expression of IFN- α , IL-12 p35, and IL-12 p40 by monocytes was examined at the mRNA level by comparative PCR analyses. It is shown in Figure 3 that activation of monocytes with LPS resulted in low levels of amplified IFN- α cDNA which was inhibited by IL-4, IL-13, and IL-10. In addition, it is shown that activation in the presence of anti-IL-10 resulted in enhanced expression of IFN- α -specific products which was also strongly inhibited by IL-4 and IL-13. Interesting, similar results were observed for the expression of IL-12 p35 and IL-12 p40. It is shown (Fig. 3) that IL-12 p35- and IL-12 p40-specific products were

Table III
Effect of IL-13, IL-4, and IL-10 on cytokine production by human monocytes^a

| Activation | Cytokine production (ng/ml) ^b | | | | | | | | | |
|--------------------------|--|--------------|--------|------|------|-------|--------|-------|--------------|----------------|
| | IL-1 α | IL-1 β | IL-1ra | IL-6 | IL-8 | IL-10 | GM-CSF | G-CSF | TNF α | MIP-1 α |
| Medium 4°C | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Medium 37°C | 0 | 0.2 | 41 | 15 | 187 | 0.9 | 0 | 0 | 0 | 0 |
| LPS | 0.49 | 3.9 | 348 | 1290 | 1260 | 30 | 0 | 7.0 | 1.8 | 115 |
| LPS + IL-4 | 0.06 | 0.8 | 809 | 271 | 244 | 5.5 | 0 | 0.4 | 0 | 4.5 |
| LPS + IL-13 | 0.05 | 0.3 | 1070 | 172 | 285 | 6.9 | 0 | 0.3 | 0 | 1.7 |
| LPS + IL-10 | 0 | 0.3 | 555 | 64 | 146 | ND | 0 | 0.3 | 0 | 0.5 |
| LPS + anti-IL-10 | 6.72 | 21.9 | 259 | 2000 | 1630 | ND | 0.52 | 179 | 16.4 | 288 |
| LPS + anti-IL-10 + IL-4 | 0.88 | 0.7 | 729 | 391 | 527 | ND | 0 | 1.1 | 0 | 25.3 |
| LPS + anti-IL-10 + IL-13 | 0.51 | 2.0 | 732 | 846 | 1330 | ND | 0.05 | 1.0 | 0.9 | 114 |

^a Human monocytes (4×10^6 /ml), purified by centrifugal elutriation, were activated by LPS (1 μ g/ml) in the presence of IL-4 (200 U/ml), IL-13 (50 ng/ml), IL-10 (200 U/ml), or anti-IL-10 mAb 19F1 (10 μ g/ml) for 20 h; and production of cytokines was measured in culture supernatants by cytokine-specific ELISA.

^b ND, not determined.

detected when monocytes were activated by LPS in the presence of anti-IL-10 mAb. This indicates that IL-10 strongly down-regulates the expression of IL-12 and that IL-12 p35 and IL-12 p40 are coordinately expressed in monocytes. In addition, IL-4 and IL-13 strongly inhibited the expression of IL-12 p35 and IL-12 p40 under these conditions.

Discussion

In the present study we determined the biologic activities of IL-13 on human monocytes. IL-13 affected the morphology, phenotype, function, and cytokine production of monocytes. We described previously that incubation of monocytes with IL-13 induced strong adherence of these cells to plastic substrates and that their morphology changed to a dendritic appearance (17). In addition, homotypic aggregates of cells were observed. The finding that IL-13 up-regulated the expression of CD11b, CD11c, CD18, VLA-5, and CD29, which are members of the integrin superfamily, is compatible with the observed aggregation of monocytes and changes in their morphology, inasmuch as CD11b/CD18 and CD11c/CD18 heterodimers are involved in cell-cell interactions, homotypic aggregation, adhesion to artificial substrates (34, 51–53), and bind fibrinogen (54). In addition, the $\alpha 5 \beta 1$ integrin VLA-5/CD29 is the receptor for fibronectin, which is an abundant extracellular matrix protein involved in adhesion processes (55). IL-13 did not induce changes in the expression of other molecules involved in adhesion or cell-cell interaction, e.g., CD11a, VLA-2, VLA3, VLA-4, VLA-6, $\beta 3$, $\beta 4$, ICAM-1, ICAM-2, LFA-3, MEL-14, and CD44, but it remains possible that other cell surface structures are involved in the IL-13-induced changes in morphology and adherence.

It has to be noted that many of the effects of IL-13 on monocyte morphology, phenotype, function, and cytokine production are shared with IL-4. IL-4 also induces strong adherence of monocytes, formation of processes, and ho-

motypic aggregation (56). In addition, IL-4 enhances class II MHC expression, inhibits ADCC activity, and cytokine production by LPS-activated monocytes (46, 48). However, because the activities of IL-13 could not be blocked by a neutralizing anti-IL-4 mAb, it is demonstrated that IL-13 acts independently of IL-4.

IL-13 down-regulated the expression of CD14. CD14 can function as a receptor for LPS and LPS-CD14 binding is increased in the presence of LPS binding protein, an unidentified serum protein (57). IL-4 also down-regulated the expression of CD14 as described (58, 59). IL-13 up-regulated the expression of CD13 (aminopeptidase-N). It has been demonstrated that IL-4 increased the expression of CD13 on mature monocytes which correlated functionally with an enhanced leucine-aminopeptidase activity (60). It can be expected that IL-13 will also increase leucine-aminopeptidase activity and that the changes in surface expression of CD13 and CD14 are part of the general monocyte-activating and differentiation-inducing characteristics of IL-13 and IL-4.

IL-13 up-regulated the expression of class II MHC Ag on human monocytes. The expression of HLA-DR, HLA-DP, and HLA-DQ was significantly increased by IL-13. IL-10 inhibited IL-13-induced class II MHC expression, which is in keeping with our previous observations indicating that IL-10 also inhibited constitutive, IL-4-, and IFN- γ -induced class II MHC expression on human monocytes (61).

The expression of the various Fc receptors for IgG and IgE on monocytes is influenced by cytokines. CD64 (Fc γ RI) expression is up-regulated by IFN- γ and IL-10, but inhibited by IL-4 (44–46, 62). Furthermore, IFN- γ and IL-10 are able to prevent the down-regulation of CD64 induced by IL-4 (44, 46). IL-13, like IL-4, inhibited the constitutive expression of CD64. This inhibitory effect was prevented by IL-10 and IFN- γ . The expression of CD64 has been shown to correlate with ADCC activity of monocytes (47). Indeed ADCC activity of monocytes incubated with

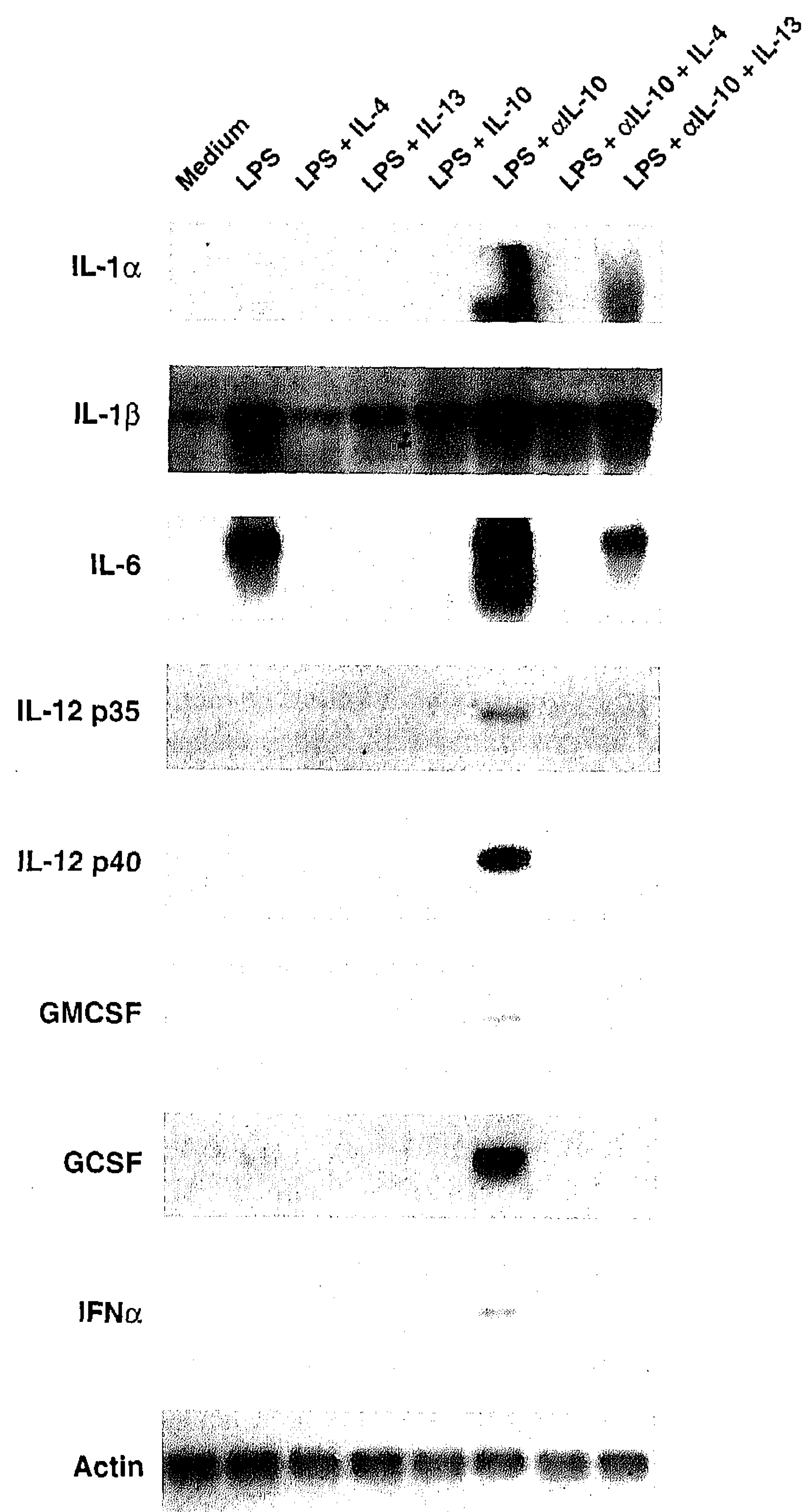


FIGURE 3. IL-13 inhibits mRNA expression of proinflammatory cytokines and hematopoietic growth factors. Elutriated monocytes ($4 \times 10^6/\text{ml}$) were activated by LPS ($1 \mu\text{g}/\text{ml}$) in the absence or presence of IL-4 (200 U/ml), IL-13 (50 ng/ml), IL-10 (200 U/ml), or combinations of these cytokines and anti-IL-10 mAb 19F1 ($10 \mu\text{g}/\text{ml}$) for 20 h. Expression of IL-1 α , IL-1 β , IL-6, IL-12 p35, IL-12 p40, GM-CSF, G-CSF, IFN α , and β -actin was determined on reverse transcribed mRNA by PCR amplification with cytokine-specific primers and detection of the reaction products by Southern analysis using internal probes.

IL-13 was strongly inhibited, indicating that IL-13 not only affected the phenotype but also the function of human monocytes. Interestingly, although IL-10 prevented the IL-13-induced down-regulation of CD64 expression, ADCC activity was still inhibited. This supports the notion that ADCC activity is not only determined by the levels of CD64 expression, but that additional, yet to be defined mechanisms play a role (44).

IL-13, like IL-4, also affected the expression of Fc γ RII

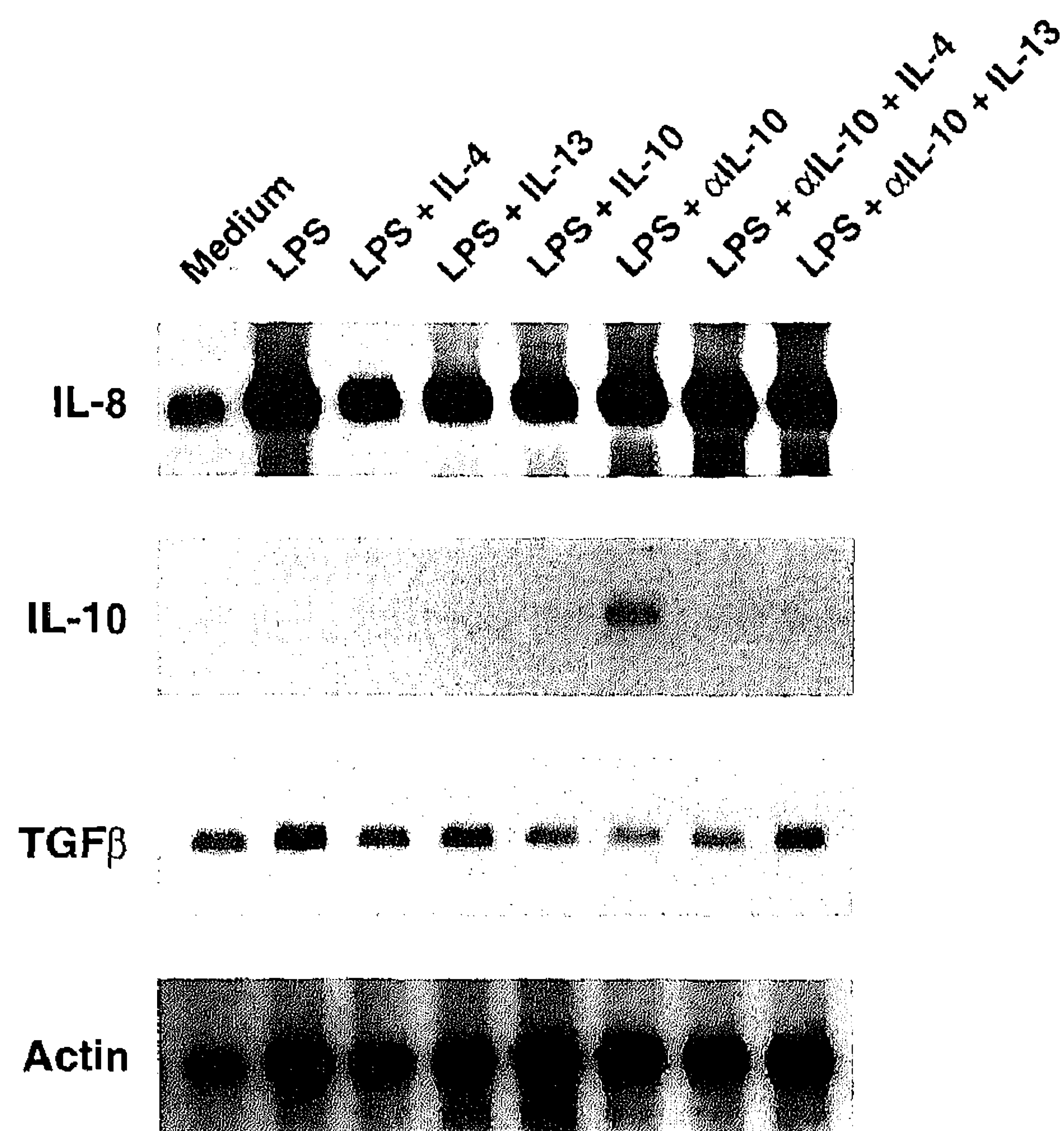


FIGURE 4. Effects of IL-13 on mRNA expression of IL-8, IL-10, and TGF β . Elutriated monocytes ($4 \times 10^6/\text{ml}$) were activated by LPS ($1 \mu\text{g}/\text{ml}$) in the absence or presence of IL-4 (200 U/ml), IL-13 (50 ng/ml), IL-10 (200 U/ml), or combinations of these cytokines and anti-IL-10 mAb 19F1 ($10 \mu\text{g}/\text{ml}$) for 20 h. Expression of IL-8, IL-10, TGF β , and β -actin was determined by Northern analysis.

and Fc γ RIII. IL-13 down-regulated the expression of CD32 and CD16 in a dose-dependent manner. However, IL-10, but not IFN- γ , could block the IL-13-induced down-regulation of CD32 and CD16 on monocytes. These results indicate that the level of Fc receptor expression is highly regulated by cytokines.

Thus far IL-4 was the only cytokine known to induce the low affinity Fc receptor for IgE (CD23) on monocytes (63). However, recently it was shown that IL-13 also induced the expression of CD23 on monocytes (17). Here it is demonstrated that the IL-13-induced expression of CD23 could be partially suppressed by IFN- γ . Like IL-4, IL-13 induces the production of IgE by PBMC (18). In addition, IL-13 initiates germline ϵ transcription in purified sIgM $^+$ B cells and induced switching to IgE production in the presence of costimulatory signals provided by activated T cell clones, T cell membranes, or transfectants expressing the CD40 ligand (18, 19). IgE production is modulated by various cytokines, including soluble CD23, which have either enhancing or inhibitory effects (64). The effects of IL-13 and IFN- γ on the expression of CD23 by human monocytes fit well within this concept.

IL-13 strongly inhibited the production of inflammatory cytokines, hematopoietic growth factors, and chemokines by LPS-activated monocytes. These activities are shared with both IL-4 and IL-10 (36, 48, 65–68). These results

further extend the observation that IL-13 inhibits the secretion of IL-6 by LPS-activated PBMC (14). Monocytes produce IL-10 after activation (36). Therefore it is important to study the effects of IL-4 and IL-13 on cytokine production in the absence of endogenously produced IL-10. Addition of a neutralizing anti-IL-10 mAb during the activation indeed resulted in strongly enhanced production of these cytokines. It is shown that IL-13, IL-4, and IL-10 strongly inhibited the production of the proinflammatory cytokines IL-1 α , IL-1 β , IL-6, IL-8, TNF α , and MIP-1 α . The inhibition of IL-1 α , IL-6, and IL-8 secretion by IL-13 was observed at both protein and steady state mRNA levels, whereas the inhibition of IL-1 β was more pronounced at the protein level. This indicated that the regulation of IL-1 β expression by cytokines also occurs post-transcriptionally, as described by Donnelly et al. (69). Of the proinflammatory cytokines, IL-8 is a member of the chemokine α subfamily and chemoattracts and activates neutrophils, whereas MIP-1 α is a member of the chemokine β subfamily which has chemoattractive and activation activities on monocytes and T cells (49, 50). On the other hand, IL-4, IL-13, and IL-10 enhance the production of IL-1ra by LPS-activated monocytes. IL-1ra is a competitive inhibitor of IL-1 α and IL-1 β and acts by binding to type I and type II IL-1 receptors without activating them (70, 71). It has been described that IL-4 induces IL-1ra by monocytes in vitro and in vivo (72–75). Through the inhibition of IL-1 activities, IL-1ra has been shown to possess anti-inflammatory activities in acute and chronic inflammation (76). Taken together, these data indicate that IL-13, IL-4, and IL-10 by inhibiting the production of proinflammatory cytokines and induction of IL-1ra may have strong anti-inflammatory activities. IL-13 also inhibited the production of the hematopoietic growth factors GM-CSF and G-CSF. This inhibition occurred at the level of gene expression. In addition it is shown that IL-13, like IL-4 and IL-10, inhibited the expression of IFN- α mRNA. This further illustrates the immunoregulatory properties of IL-13.

IL-13, like IL-4, inhibited the production of IL-10 mRNA expression. Inhibition of IL-10 production by these Th2 cytokines may play a role in the establishment of chronic disease states because IL-10 has been identified as a general immune suppressor by down-regulating the production of inflammatory cytokines, class II MHC expression on monocytes and T cell and NK cell responses (77).

Recently, it has been described that IL-12, a heterodimeric cytokine composed of 35-kDa and 40-kDa polypeptides is produced by EBV-transformed B cell lines and *Staphylococcus aureus* Cowan I-activated monocytes (78). Here, we have shown that monocytes activated by LPS in the presence of neutralizing anti-IL-10 mAb can also transcribe IL-12 p35 and IL-12 p40 mRNA and that this expression is strongly inhibited by IL-13, IL-4, and IL-10. The expression of IL-12 p40 chain mRNA seemed to be

more abundant than IL-12 p35 in accordance with data from D'Andrea et al. (78). IL-12 has been shown to induce and enhance the expression of IFN- γ by T cells and NK cells (79). The inhibitory effects of IL-4, IL-13, and IL-10 on the production of IL-12 could play a role in the inhibitory effects of these cytokines on the production of IFN- γ , a possibility which we are currently investigating. Inhibition of IL-12 by IL-4, IL-13, and IL-10 could also affect the cross-regulation of Th1 and Th2 subsets because IL-12 has recently been shown to play a role in the development of Th1 cells (80, 81).

IL-13 shares many of its activities with IL-4. Both cytokines induce similar changes in cell surface phenotype, morphology, function, and cytokine production by human monocytes. Adding combinations of saturating amounts of IL-4 and IL-13 to monocytes resulted in identical changes in phenotype as compared with those induced by either cytokine alone. In addition, IL-13, like IL-4, is able to induce the production of IgE by human B cells (18, 19). However, unlike IL-4, IL-13 does not support the proliferation of PHA blasts or T cell clones (R. de Waal Malefyt, unpublished data) nor does it induce the expression of CD8 α on CD4 $^{+}$ T cell clones or cord blood T cells (H. Yssel and H. Spits, unpublished data). Recently, Zurawski et al. have shown that IL-13 was able to competitively inhibit binding of IL-4 to IL-4R on TF-1 cells but not on Ba/F3 cells that were transfected with the IL-4R-binding protein (82). In addition, an IL-4 mutant protein which binds, but does not activate the IL-4 receptor, has antagonistic activities and blocked both IL-4- and IL-13-induced biologic activities (82). These results indicate that IL-4R and IL-13R share a receptor component necessary for signal transduction which is not the IL-4R-binding protein. The multiple biologic activities of IL-13 on monocytes indicate that this factor could play an important role in inflammatory, allergic, and immunoregulatory processes.

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